

Amendments to the Claims:

Claim 1 (Original): A method for generating hydroxylated 14-membered macrolide compounds said method comprising:

- (a) producing a 14-membered aglycone template; and,
- (b) feeding said aglycone template to a strain capable of hydroxylating the aglycone template at the 14 and/or 15 position.

Claim 2 (Original): The method of claim 1, wherein the strain is identified by screening a library of prokaryotes and fungal strains to identify those which are capable of hydroxylating the aglycone template at the 14 and/or 15 position.

Claim 3 (Original): The method of claim 2, wherein the strain is identified by screening a library of actinomycetes.

Claim 4 (Original): The method of claim 1, wherein the strain is selected from the group consisting of *Streptomyces eurythermus*, *Streptomyces avermitilis* and *Streptomyces rochei*.

Claim 5 (Original): The method of claim 1, wherein the strain is selected from the group consisting of *Streptomyces eurythermus* DSM 40014, *Streptomyces avermitilis* ATCC 31272 and *Streptomyces rochei* ATCC 21250.

Claim 6 (Previously Presented): The method of claim 1, wherein the strain used in step (b) is genetically engineered to express a cytochrome P450 capable of hydroxylating the starter unit region of the aglycone template.

Claim 7 (Original): The method according to claim 6, wherein the recombinant strain used in step (b) is a prokaryote.

Claim 8 (Original): The method according to claim 7, wherein the recombinant strain used in step (b) is *E. coli*.

Claim 9 (Original): The method according to claim 7, wherein the recombinant strain used in

step (b) is an actinomycete.

Claim 10 (Original): The method according to claim 9, wherein the recombinant strain used in step (b) is selected from the group consisting of *Saccharopolyspora erythraea*, *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Streptomyces griseofuscus*, *Streptomyces cinnamonensis*, *Streptomyces fradiae*, *Streptomyces eurythermus*, *Streptomyces longisporoflavus*, *Streptomyces hygroscopicus*, *Saccharopolyspora spinosa*, *Micromonospora griseorubida*, *Streptomyces lasaliensis*, *Streptomyces venezuelae*, *Streptomyces antibioticus*, *Streptomyces lividans*, *Streptomyces rimosus*, *Streptomyces albus*, *Amycolatopsis mediterranei*, *Nocardia sp*, *Streptomyces tsukubaensis* and *Actinoplanes sp. N902-109*.

Claim 11 (Previously Presented): The method of claim 1 wherein said hydroxylated 14-membered aglycone product is isolated after step (b).

Claim 12 (Previously Presented): The method of claim 1 which additionally comprises the step of

(c) feeding the resulting hydroxylated 14-membered aglycone to a second strain which is able to add one or more sugar moieties.

Claim 13 (Original): The method of claim 12 wherein said hydroxylated aglycone produced is fed directly to the strain of step (c) with no purification step.

Claim 14 (Previously Presented): The method of claim 12 wherein the second strain naturally synthesises the desired sugar moiety or moieties and is capable of adding them to the hydroxylated 14-membered aglycone template.

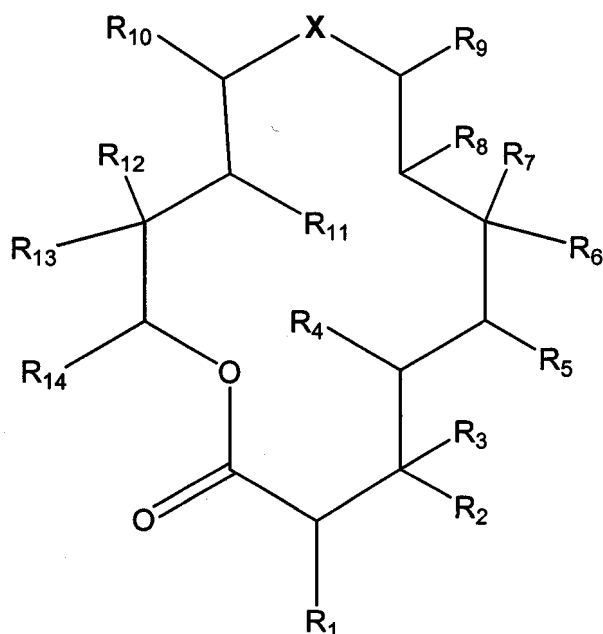
Claim 15 (Previously Presented): The method of claim 12, wherein the second strain is genetically engineered to express and / or transfer the desired sugar moiety or moieties.

Claim 16 (Original): The method of claim 15, wherein the method of genetically engineering the strain comprises introducing into said strain gene cassette(s) containing the biosynthetic genes responsible for the synthesis and / or transfer of the desired sugar moiety or moieties.

Claim 17 (Previously Presented): The method according to claim 12, wherein the strain used in step (c) is an actinomycete.

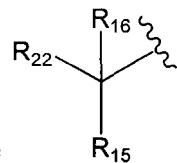
Claim 18 (Original): The method according to claim 17, wherein the strain used in step (c) is selected from the group consisting of *Saccharopolyspora erythraea*, *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Streptomyces griseofuscus*, *Streptomyces cinnamonensis*, *Streptomyces fradiae*, *Streptomyces eurythermus*, *Streptomyces longisporoflavus*, *Streptomyces hygroscopicus*, *Saccharopolyspora spinosa*, *Micromonospora griseorubida*, *Streptomyces lasaliensis*, *Streptomyces venezuelae*, *Streptomyces antibioticus*, *Streptomyces lividans*, *Streptomyces rimosus*, *Streptomyces albus*, *Amycolatopsis mediterranei*, *Nocardia sp.*, *Streptomyces tsukubaensis* and *Actinoplanes sp. N902-109*.

Claim 19 (Previously Presented): The method according to claim 1 wherein the aglycone template fed to said strain in step (b) is according to the formula below:

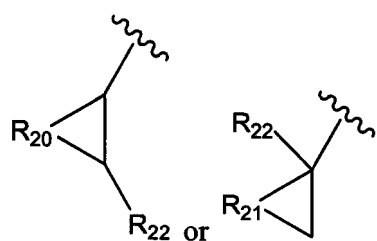
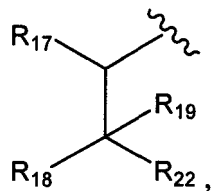


Where:

X = -C(=O)-, -CH(OH)- or -CH₂-, R₁, R₄, R₆, R₉, R₁₀ and R₁₂ are each independently H, OH, CH₃, CH₂CH₃ or OCH₃; R₂ = OH; R₃ = H; or R₂ and R₃ together are keto; R₅ = OH; R₇ = H,



OH or OCH₃; R₈ = H, OH or keto; R₁₁ = H, OH; R₁₃ = H, OH, and R₁₄ =

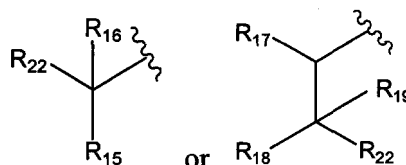


where: R₁₅ is H or a C₁-C₇ alkyl group or C₄-C₇ cycloalkyl

group; R₁₆ is H, a C₁-C₇ alkyl group or C₄-C₇ cycloalkyl group, R₁₇, R₁₈ and R₁₉ are each independently H or a C₁-C₇ alkyl group or R₂₀ or R₂₁ are (CH₂)_x where x = 2-5 and R₂₂ is H; or a variant of a compound as defined above modified by replacing one or more >CHOH or >CHOMe groups by a keto group, or variant of a compound as defined above which differs in the oxidation state of one or more of the ketide units (i.e. selection of alternatives from the group: -CO-, -CH(OH)-, alkene -CH- (=CH- or -CH=), and CH₂).

Claim 20 (Original): The method of claim 19, wherein

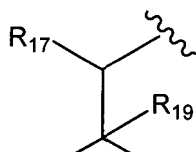
X = -C(=O)-, R₁=R₄ = R₆ = R₉ = R₁₀ = R₁₂ = CH₃, R₂ = OH, R₇ = H, OH; R₈ = H, OH,



OCH₃; R₁₁ = H, OH; R₁₃ = H, OH; R₁₄ = or R₁₈ and R₂₂ , where: R₁₅ = H, CH₃, or CH₂CH₃ and R₁₆ is H; or R₁₇ and R₁₈ are each independently H or CH₃; R₁₉ and R₂₂ are H.

Claim 21 (Previously Presented): The method of claim 19, wherein:

X = -C(=O)-, R₁, R₄, R₆, R₉, R₁₀ and R₁₂ are each CH₃, R₂, R₅ and R₁₁ = OH; R₃, R₈ and R₁₃=



H; R₇ = H or OH, and R₁₄ = R₁₈ R₂₂ where: R₁₇, R₁₈, R₁₉ and R₂₂ = H.

Claim 22 (Original): The method according to claim 6, wherein the oxidative enzyme is identified by screening a library of prokaryotic and fungal strains and cloning the range of oxidative enzymes expressed within a strain capable of hydroxylating the 14-membered aglycone template at the 14 and/or 15 position.

Claim 23 (Original): The method according to claim 22, wherein the library screened is a library of actinomycetes.

Claim 24 (Previously Presented): The method according to claim 22, wherein the range of oxidative enzymes within the strain identified as capable of hydroxylating the 14-membered aglycone template at the 14 and/or 15 position are identified using degenerate oligo primers.

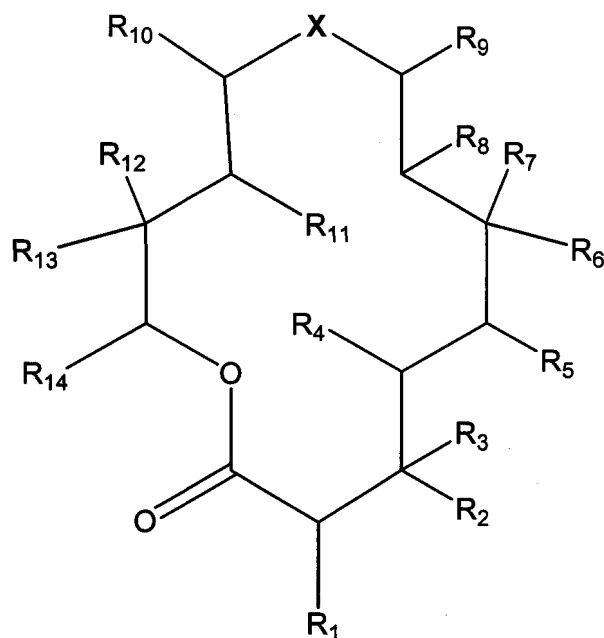
Claim 25 (Previously Presented): The method according to claim 22 wherein the oxidative enzyme(s) is a cytochrome P450.

Claim 26 (Original): A method for generating hydroxylated 14-membered macrolide compounds said method comprising:

- (a) producing a 14-membered aglycone template,
- (b) identifying a cytochrome P450 capable of hydroxylating the 14-membered aglycone template at the 14 and/or 15 position by screening a library of prokaryotic and fungal strains and amplifying the range of P450s expressed within a strain,
- (c) expressing and isolating said P450, and
- (d) using the isolated P450 in vitro to hydroxylate the 14 and/or 15 position of said 14-membered aglycone template.

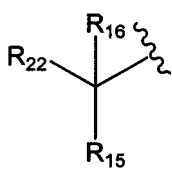
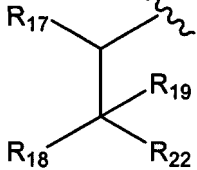
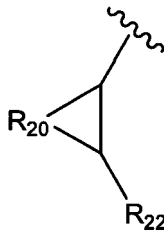
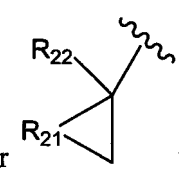
Claim 27 (Original): The method of claim 26, wherein said P450 is expressed together with appropriate ferredoxin and ferredoxin reductases.

Claim 28 (Previously Presented): A process according to claim 1 which produces one or more compounds according to formula I:



Where:

X = -C(=O)-, -CH(OH)- or -CH₂-, R₁, R₄, R₆, R₁₀ and R₁₂ are each independently H, OH, CH₃, CH₂CH₃ or OCH₃; R₂ = OH, or any glycosyl or disaccharide group, R₃ = H; or R₂ and R₃ together are keto; R₅ = OH, or any glycosyl group, R₇ = H, OH, OCH₃; R₈ = H, OH or keto; R₉ = H, OH, CH₃, CH₂CH₃ or OCH₃, *O*-megosamine, *O*-cladinose, *O*-mycarose, *O*-rhamnose or a methylated derivative thereof, *O*-digitoxose, *O*-olivose, *O*-oliose or *O*-oleandrose; *O*-desosamine, *O*-mycaminose or *O*-angolosamine; R₁₁ = H, OH; R₁₃ = H, OH,

and R₁₄ = , ,  or  where: R₁₅ is H or a C₁-C₇ alkyl group or C₄-C₇ cycloalkyl group; R₁₆ is H, a C₁-C₇ alkyl group or C₄-C₇ cycloalkyl group, R₁₇, R₁₈ and R₁₉ are each independently H or a C₁-C₇ alkyl group or R₂₀ or R₂₁ are (CH₂)_x where x = 2-5 and R₂₂ is O-R₂₃ where R₂₃ = H or a C₁ to C₇ alkyl group or C₁-C₇ acyl group; or R₂₂ and R₁₆ together are a keto group; or R₂₂ and R₁₉ together are a keto group; or a variant of a compound as defined above which differs in the oxidation state of one

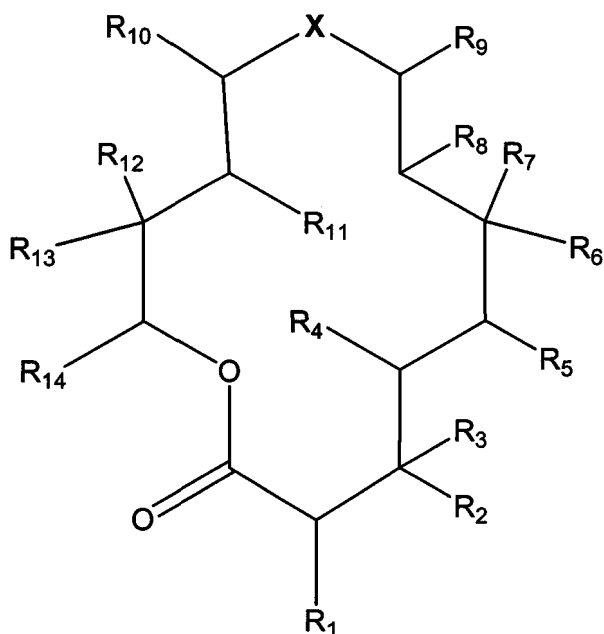
or more of the ketide units (i.e. selection of alternatives from the group: -CO-, -CH(OH)-, alkene -CH- (=CH- or -CH=), and CH₂).

Claim 29 (Original): A process according to claim 28 wherein R₂ is selected from *O*-cladinose, *O*-mycarose, *O*-rhamnose and methylated derivatives thereof, *O*-digitoxose, *O*-olivose, *O*-oliose or *O*-oleandrose.

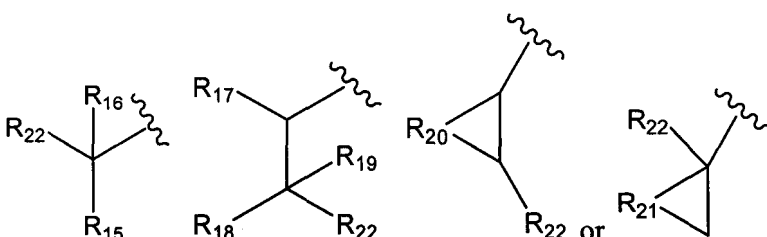
Claim 30 (Original): A process according to claim 29 wherein R₂ and/or R₉ is a said methylated derivative selected from 2'-*O*-methyl, 2',3'-*bis-O*-methyl and 2',3',4'-*tris-O*-methyl.

Claim 31 (Previously Presented): A process according to claim 28, wherein R₅ is a glycosyl group selected from *O*-mycaminose and *O*-angolosamine.

Claim 32 (Currently Amended): A compound according to formula I below:

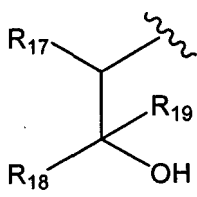


wherein X = -C(=O)-, -CH(OH)- or -CH₂-, R₁, R₄, R₆, R₉, R₁₀ and R₁₂ are each independently H, CH₃ or CH₂CH₃, R₂ = OH or any glycosyl group; R₃ = H, or R₂ and R₃ together are keto; R₅ = OH or any glycosyl group; R₇ = H, OH, ~~OCH₃~~; R₈ = H, OH; R₁₁ = H, OH, R₁₃ = H, OH,



 $R_{14} =$, R_{18} , R_{22} or where: R_{15} is H or a C_1 - C_7 alkyl group or C_4 - C_7 cycloalkyl group; R_{16} is H, a C_1 - C_7 alkyl group or C_4 - C_7 cycloalkyl group, R_{17} , R_{18} and R_{19} are each independently H or a C_1 - C_7 alkyl group or R_{20} or R_{21} are $(CH_2)_x$ where $x = 2-5$ and R_{22} is $O-R_{23}$ where $R_{23} = H$ or a C_1 to C_7 alkyl group or C_1 - C_7 acyl group; ~~or $R_{22} = \text{halogen or } NR_{24}R_{25}$, where R_{24} and R_{25} are each independently H, a C_1 to C_7 alkyl group or C_1 - C_7 acyl group;~~ or R_{22} and R_{16} together are a keto group; or R_{22} and R_{19} together are a keto group; or a variant of a compound as defined above which differs in the oxidation state of one or more of the ketide units (i.e. selection of alternatives from the group: $-CO-$, $-CH(OH)-$, alkene $-CH-$, and CH_2); with the proviso that the following compounds are excluded:

- (a) when $R_2 = OH$, *O*-cladinose or *O*-mycarose and R_5 is OH or *O*-desosamine
- (b) when $R_1 = R_4 = R_6 = R_9 = R_{10} = R_{12} = CH_3$, $R_3 = H$, $R_2 = O$ -oleandrose, $R_5 =$



O-desosamine, $R_7 = OH$, $R_8 = R_{13} = H$ and $R_{14} = R_{18}$, where $R_{17} = R_{18} = R_{19} = H$,

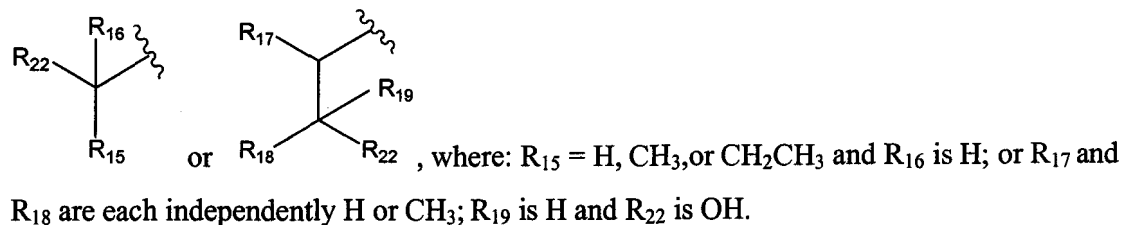
- (c) when R_2 or $R_5 = O$ -mycaminose
- (d) when R_2 or $R_5 = O$ -angolosamine.

Claim 33 (Original): A compound according to claim 32 wherein R_2 is selected from *O*-cladinose, *O*-mycarose, *O*-rhamnose and methylated derivatives thereof, *O*-digitoxose, *O*-olivose, *O*-oliose or *O*-oleandrose.

Claim 34 (Original): A compound according to claim 33 wherein R_2 is a said methylated derivative selected from 2'-*O*-methyl, 2',3'-*bis-O*-methyl and 2',3',4'-*tris-O*-methyl.

Claim 35 (Previously Presented): A compound according to claim 32, wherein R_5 is a glycosyl group selected from *O*-mycaminose and *O*-angolosamine.

Claim 36 (Previously Presented): A compound according to claim 32, where $X = -C(=O)-$, $R_1 = R_4 = R_6 = R_9 = R_{10} = R_{12} = CH_3$, $R_2 = OH$, *O*-rhamnose or a methylated derivative thereof, *O*-digitoxose, *O*-olivose, *O*-oliose or *O*-oleandrose, $R_3 = H$, $R_5 = OH$, *O*-mycaminose or *O*-angolosamine; $R_7 = H, OH$; $R_8 = H, OH, OCH_3$; $R_{11} = H, OH$; $R_{13} = H, OH$; $R_{14} =$



Claim 37 (Original): A compound according to claim 36, where $X = -C(=O)-$, $R_1 = R_4 = R_6 = R_9 = R_{10} = R_{12} = CH_3$, $R_2 = OH$, *O*-rhamnose or a methylated derivative thereof, *O*-digitoxose, *O*-olivose, *O*-oliose or *O*-oleandrose; $R_3 = H$; $R_5 = OH$, *O*-mycaminose or *O*-angolosamine; $R_7 = H, OH$; $R_8 = H, OH, OCH_3$; $R_{11} = H, OH$; $R_{13} = H, OH$; $R_{14} =$

